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Clinical evaluation of multiplex RT-PCR assays for the detection of influenza A/B and respiratory syncytial virus using a high throughput system

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ABSTRACT

Background: Lower respiratory tract infections are a major threat to public health systems worldwide, with RSV and influenza being the main agents causing hospitalization. In outbreak situations, high-volume respiratory testing is needed. In this study, we evaluated the analytical and clinical performance of a pre-designed primer/probe set for the simultaneous multiplex detection of both viruses on a high-throughput platform, the cobas® 6800, using the "open channel" of the system for integration of lab-developed assays for the detection of influenza and RSV.

Results: Using the influenza/RSV qPCR Assay with swabs, LoD (95%) in TCID50/mL for influenza-A was 0.009, influenza-B 0.003, RSV-A 0.202, and RSV-B 0.009. Inter-run variability (3xLoD) was low (< 1 Ct for all targets). Of 371 clinical respiratory specimens analyzed, results were concordant for 358 samples. The calculated sensitivity and specificity of the assay were 98.3% and 98.4% for Flu-A, 100% and 98.5% for Flu-B, and 98.6% and 99.7% for RSV. All quality assessment panel specimens (N = 63, including avian influenza strains) were correctly identified. None of the tested microorganisms showed cross-reactivity.

Conclusion: Compared with CE-IVD assays, the assay evaluated here showed good analytical and clinical sensitivity and specificity with broad coverage of different virus strains. It offers high-throughput capacity with low hands-on time, facilitating the laboratory management of large respiratory outbreaks.

1. Introduction

Influenza viruses type A and B (influenza A/B) and respiratory syncytial virus (RSV) are important causes of morbidity and mortality from lower respiratory tract infections (LRTI) in children (Malosh et al., 2018; Robinson, 2008), the elderly (Branche and Falsey, 2015; Falsey et al., 2008; Malosh et al., 2018) and immunocompromised patients (Hakim et al., 2016; Martino et al., 2005; Waghmare et al., 2013). The clinical symptoms of influenza and RSV are non-specific and it is difficult to distinguish between the two infections based on clinical symptoms alone (CDC, 2018c). Nevertheless, accurate diagnosis of respiratory illnesses is necessary for timely antiviral treatment, restricting antibiotic use, and facilitating infection control and prevention strategies (Rappo et al., 2016; Rogan et al., 2017; Schulert et al., 2013; Xu et al., 2013).

RSV, the most common cause of childhood RTIs, is a major cause of hospitalization, and a leading cause of childhood death from RTIs (Nair et al., 2010). A recent meta-analysis estimated that in 2015 there were 33.1 million episodes of acute LRTI due to RSV globally in children under 5 years, resulting in 3.2 million hospital admissions and 59,600 deaths (Shi et al., 2017). In the elderly, surveillance studies have found that 5–10% of those attending adult day-care facilities develop RSV infection annually (Falsey et al., 2008).

The World Health Organization (WHO) estimates there are 3–5 million cases of severe influenza annually resulting in 290,000–650,000 deaths worldwide (WHO, 2018b). The most recent WHO fact sheet of top 10 causes of death listed lower respiratory infections at number 4. According to WHO, lower RTIs remained the most deadly communicable disease, causing 3.0 million deaths worldwide in 2016 (WHO, 2018c).

The yearly variation in influenza illnesses poses a high burden of testing on the diagnostic molecular lab (Mathews et al., 2009; WHO, 2018a). The sudden emergence of a novel influenza A virus in 2009 resulting in a declaration of a Public Health Emergency of International

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Concern (PHEIC) (Cheng et al., 2012) and the 2017/18 influenza season estimated to have caused over 48.4 million illnesses and 79,400 deaths (CDC, 2018a; ECDC, 2018), illustrate the need for surge capacity testing. The cobas® 6800/8800 System can manage high-throughput molecular testing (up to 960 results/8-hour shift) (FDA, 2014) but currently there are no tests for respiratory infections available on this system. The objective of this study was to evaluate the analytical and clinical performance of a Flu-A/B + RSV multiplex assay using the cobas omni Utility Channel, an open channel on the cobas® 6800/8800 System.

2. Material and methods

2.1. Study design

All clinical samples and panels were assessed using Influenza/RSV qPCR. Assay (Integrated DNA Technologies, Inc. [IDT], Illinois, USA) and generic cobas omni Utility Channel Reagent Kit tested with the cobas omni Utility Channel on the cobas 6800/8800 System (UCT). Analytical performance evaluation included limit of detection (LoD), inter-run variability, testing of external quality assessment panels, and testing of potential cross-reacting micro-organisms. Clinical performance was assessed comparing the UCT with current routine diagnostic assays (AmpliGnost VolkmanPIIM, Karlsruhe, Germany and RealStar RSV RT-PCR, Altona, Hamburg, Germany).

2.1.1. LoD and inter-run variability

LoD for the UCT was determined for Influenza A, Influenza B, RSV A and RSV B. For each target, three independent dilution series and 21 replicates were assessed. Dilution series comprised six concentrations 4×, 2×, 1×, 0.5×, 0.25× and 0.125× LoD and one negative sample. Either influenza A and RSV Subtype A or influenza B and RSV Subtype B spiked in Universal Transport Medium (UTM, Copan, Brescia, Italy) was used. For inter-run variability two positive concentration levels (at 3× and 10× LoD) and one negative member was tested. There were three runs, each comprising five replicates per panel member. The intra-run variability of the assay was calculated from the mean cycle threshold (Ct) of all panel members at each concentration level and the respective standard deviation (SD).

2.1.2. External quality assessment panels and potential cross-reacting micro-organisms

To assess the quality and inclusivity of the UCT assay, external quality assessment panels for influenza A/B and RSV were tested using Quality Control for Molecular Diagnostics (QCMD) 2016, External Quality Assessment Program (Qnostics; Glasgow, UK) and Instand (Dusseldorf, Germany). For cross-reaction testing, a panel of bacteria and viral strains (n = 28) were used to assess analytical specificity and potential interference (for detailed information see Supplementary Table 1). The organisms were spiked into UTM contrived media and testing was performed in the presence or absence of influenza A, influenza B and RSV target (at approximately 3× LoD). Cross-reacting micro-organisms were evaluated by agreement calculation between observed and expected results.

Table 2
Assessment of the Limit of Detection (LoD) for influenza A, influenza B, RSV A and RSV B using three independent dilution series and 21 replicates for each target.

	LoD (probit) [TCID50/mL]	95% CI (lower, upper) [TCID50/mL]
Flu-A	0.009	0.0073–0.0138
Flu-B	0.003	0.0023–0.0048
RSV A	0.202	0.1692–0.3327
RSV B	0.009	0.0076–0.0132

CI, Confidence Interval; LoD, Limit of detection; mL, milliliter; Flu-A, influenza A; Flu-B, influenza B; RSV, Respiratory Syncytial Virus; TCID50, 50% Tissue Culture Infective Dose.

2.1.3. In silico analysis of the inclusivity of the primer and probes

To test inclusivity of the primers, models were used to predict dCt – a delay in qPCR signal compared to perfectly matching primers (pm). The algorithm was based on experiments performed with 20 perfect match primers and 268 matching mismatch (mm; 1–6 mismatches) using both DNA and RNA templates to experimentally measured delay in qPCR signal: dCt = Ct_mm – Ct_pm values. dCt predicted for an influenza isolate that has mismatches under primers is a sum of dCt_DNA for the forward primer and dCt_RNA for the reverse one. The models use thermodynamics parameters (free energies, dG) for DNA:DNA interactions (SantaLucia and Hicks, 2004). The melting temperatures for probes (Tm) were calculated using Melting5 software (Dumousseau et al., 2012) at 100 nM probe concentration, 1.7 mM Mg^{2+} and 50 mM Na/K^{+}.

2.1.4. Sensitivity and specificity

A total of N = 371 clinical specimens were analyzed, to ensure testing of at least 20 positive samples and 200 negative samples per target. The UCT results were compared with influenza (AmpliGnost Influenza A/B) and RSV (RealStar RSV RT-PCR) Conformité Européene in vitro diagnostic (CE-IVD) molecular tests. Specimens showing discordant results in the experimental assay on the UCT versus the routinely used assay were analyzed using a third PCR-assay (Xpert Flu/RSV XC, Cepheid) (for detailed information see Supplementary Methods 1.1 and 1.2).

Table 1
PCR cycling conditions and settings in the Utility Channel software used to create the run template.

Software settings:					
Sample type	Swab sample (400 µL input)				
Channels	1: none	2: Influenza A	3: Influenza B	4: RSV	IC
RFI		1.3	2	1.2	Predefined
PCR cycling conditions					
	UNG incubation	Pre-PCR step	1st measurement	2nd measurement	Cooling
No. of cycles	Predefined	1	5	45	Predefined
No. of steps		3	2	2	
Temperature		55 °C; 60 °C; 65 °C	95 °C; 55 °C	91 °C; 58 °C	
Hold time		120 s; 360 s; 240s	5s; 30s	5s; 25s	
Data acquisition		None	End of each cycle	End of each cycle	

UNG, uracil-DNA N-glycosylase; PCR, polymerase chain reaction; s, seconds.

Table 3
Inter-run variability determined by Mean Ct and standard deviation for influenza A, influenza B, RSV A and RSV B assessed using the multiplex assay on the UCT at two LoD concentrations.

Panel member (conc.)	Ct-Influenza A	Ct-Influenza B	Ct-RSV A	Ct-RSV B
10× LoD ± SD	34.32 ± 0.22	36.06 ± 0.38	35.63 ± 0.51	37.27 ± 0.60
3× LoD ± SD	35.72 ± 0.41	38.39 ± 1.14	37.03 ± 0.30	38.72 ± 0.97
neg	neg	neg	neg	neg

Ct, cycle threshold; LoD, limit of detection; neg, negative; SD, standard deviation; RSV, Respiratory Syncytial Virus.

Table 4
In silico predictions for influenza A and influenza B assays for 2018 GISAID sequences. The number of sequences is shown where mismatches of oligonucleotides can affect qPCR performance. dCt is predicted by qPCR signal delay due to mismatches (mm) under primers compared to perfectly matching (pm) primers. Also shown is the distribution of predicted Tm for probes with mismatches.

dCt due to primer mm	Flu-A-2018 #sequences, %		Flu-B-2018 #sequences, %	
dCt = 0 (pm)	6414	96.6%	2632	89.7%
0 < dCt < 5	30	0.5%	169	5.8%
5 < dCt < 10	197	3.0%	131	4.5%
dCt > 10	2	0.0%	2	0.1%
Probe Tm				
perfect_match	3129	47.1%	2728	93.0%
Tm > 68 °C	3445	51.9%	204	7.0%
65 °C < Tm < 68 °C	58	0.9%	2	0.1%
62 °C < Tm < 65 °C	11	0.2%	0	0.0%
Total	6643	100.0%	2934	100.0%

mm, mismatches; pm perfectly matching; Tm, melting temperature.

2.2. Samples and ethics

External quality assessment panels were obtained from Qnostics (Glasgow, UK), Instand (Dusseldorf, Germany). Cell culture fluids (influenza B) were obtained from Zeptometrix (Buffalo, NY, USA). All samples were stored at ≤-18 °C, or according to the package insert, prior to testing. For determination of LoD, inter-run variation and testing of cross-reactivity of organisms in contrived sample matrix (consisting of UTM media, 0.08333% Mucin and 5×10^4 HCT-15 cells) were used. Standard target positive material for Influenza A/B and RSV as well as the panel of bacteria and viruses used for analytical specificity and potential interference were obtained from Zeptometrix, USA and ATCC, RMSCC (Roche Culture Collection), and Roche Penzberg as stated in Supplementary Table 1. Anonymized clinical specimens (nasopharyngeal swabs in UTM were obtained from BioCollections Worldwide Inc., (BCW, Miami, Florida, USA) and under IRB-approved RMS protocol FR4, Rev 3.0, Multi-Center Study of In Vitro Diagnostic Devices for the Detection of Influenza A, Influenza B, and RSV.

2.2.1. Flu-A/B+RSV on the UCT

Sample preparation as well as amplification/detection for the UCT were carried out on the cobas 6800 System. The primer/probe set as well as test conditions (sample preparation, thermocycling profile) were provided by IDT, and are available from IDT via the following link https://www.idtdna.com/pages/products/reagents-and-kits/microbial-detection/microbial-identification-assays. The targets for the probes are as follows: Flu-A and Flu-B: segment 7, matrix protein 1 (M1); RSV: RSV matrix protein (M).

Briefly, 480 µL of the IDT primer–probe-mix was added to 8 mL Roche Master Mix (MMx-2), then 7 mL of the primer–probe/MMx-2 mix were transferred to the reagent cassette as recommended by the manufacturer (Cobb et al., 2017). The settings in the cobas Utility Channel software and cycling conditions used in this study are shown in Table 1.

2.3. Data analysis and software

LoD was determined by probit analysis including 95% Confidence Intervals (CIs). All statistical analyses were performed using SAS JMP 12.1.0. Cobas 6800 software 1.2, and cobas omni Utility Channel software 3.0 was used in this study.

3. Results

3.1. Limit of detection of the influenza A/B + RSV assay

In total, 63 replicates per concentration level were tested. The probit LoD (95% CI) of the influenza A/B + RSV assay for nfluenza A, influenza B, RSV A and RSV B was 0.009 (0.0073–0.0138), 0.003 (0.0023–0.0048), 0.202 (0.1692–0.3327) and 0.009 (0.0076–0.0132) TCID50/mL, respectively (Table 2).

3.2. Inter-run variability

Three independent runs, each comprising 5 replicates per panel member were tested. Inter-run variability was low, less than 0.6 Ct standard deviation at 37.25 Ct (10× LoD) and less than 1.1 Ct standard deviation at 38.39 at 3× LoD; respectively (Table 3) demonstrating high reproducibility of the assay.

3.3. Cross-reactivity

Assessment of analytical specificity and potential interference against a panel of 28 bacteria and viruses showed that none of the organisms tested showed false positive results in the UCT assay. Detection of influenza and RSV were not affected by the presence of any of the organisms tested (Δ Ct less than 1).

3.4. In silico analysis

In silico analysis shows *in silico* predictions for Flu-A and Flu-B tests in 6,643 and 2,934 sequences, respectively. All sequences were collected from human subjects in 2018 and were downloaded from GISAID on December 11, 2018 (Shu Y, McCauley J. 2017). The dataset includes 3,169 H1N1 and 3,457 H3N2 Flu-A subtypes. Table 4 shows that for 96.6% / 89.7% of Flu-A / Flu-B GISAID sequences primers match perfectly. Only two sequences for each virus are predicted to be delayed by > 10 cycles; in case of Flu-A these two are H9N2 and H7N9 subtypes collected in China in January 2018. Table 4 also shows the number of GISAID sequences where probes have mismatches binned by predicted probe melting temperatures (Tm). The probes were designed to have a high Tm, so that they can tolerate mismatches. In case of Flu-A the majority of sequences have two mismatches against the Flu-A probe, resulting in predicted Tm = 69.4 °C. In our experience probes with Tm > 68 °C shows signal similar to pm probes; probes with 65° < Tm < 68 °C give sufficient signal to detect viruses and probes with 62° < Tm < 65 °C give signal that may be too low to make positive calls.

In addition, 43 avian Flu-A H5 2018 sequences from GISAID were analyzed: 11H5N6 isolates have no mismatches under the primers; 28

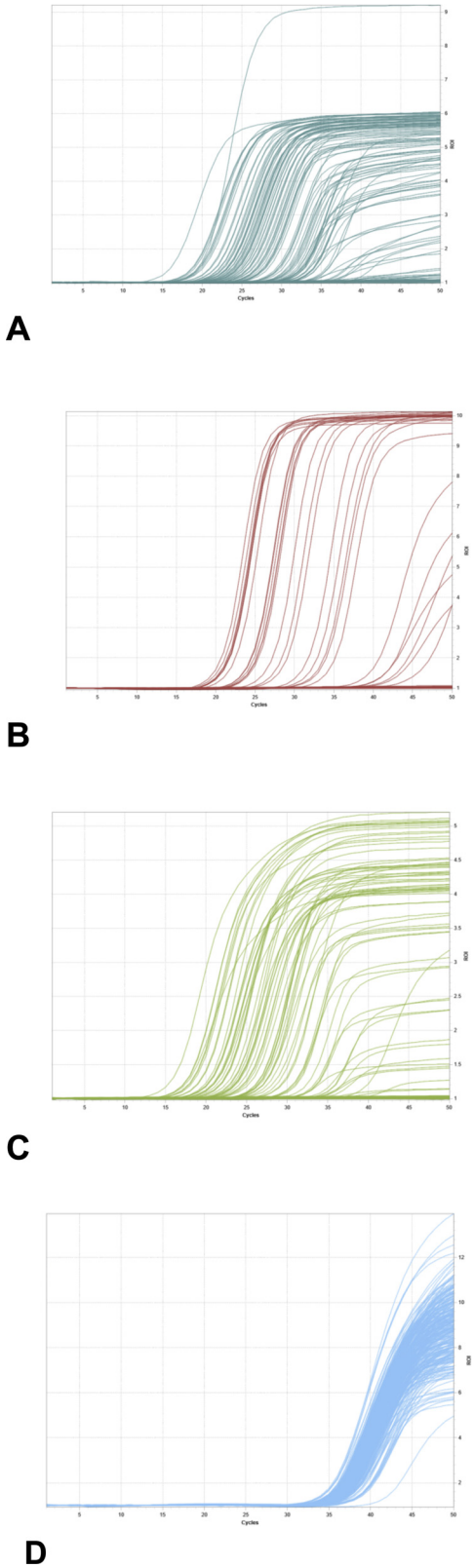

Fig. 1. PCR amplification curves of all clinical samples tested with the UCT assay (n = 371) A: influenza A; B: influenza B; C: RSV; D: internal control.

H5N8 have predicted dCt˜6 cycles; one H5N1 has dCt˜4 cycles; another is a perfect match. For all 43 H5 isolates Tm_probe > 71 °C. Thus, our prediction is that the Flu-A test will detect 2018 avian H5 isolates.

3.5. External qualification panels

To assess the quality and inclusivity of the UCT assay external quality assessment panels for influenza A/B and RSV were applied. For influenza, 10 samples (Qnostics, Supplementary Table 2) and 41 samples (Instand, Supplementary Table 3) were tested. These panels contain positive samples with the following typical influenza A strains: H1N1_09, H1N1, H3N2, as well as avian influenza A strains such as H5N1, H5N8, H7N9 and influenza B strains from the Brisbane and the Yamagata lineage. All members (51/51) of the influenza panels were detected correctly. As predicted by the *in silico* analysis, samples with H7N9 showed high Ct values and amplification curves (see Supplementary Table 3) with low region of interest (ROI) values (data not shown). Additionally, for RSV, eight samples (Qnostics, QMC panel 2016) and four samples (Instand panel) were analyzed. These panels contain positive samples from RSV lineage A and B (Supplementary Tables 2 and 3). All members of the RSV panels were detected correctly. In total, 63/63 panel samples were correctly identified and reported as valid results, indicating a broad, sensitive, and robust coverage of the current circulating or possibly emerging strains.

3.6. Sensitivity and specificity

To evaluate clinical performance, the UCT assay was compared with CE-IVD influenza and RSV molecular tests routinely used in the Heidelberg laboratory (AmpliGnost Influenza A/B; RealStar RSV RT-PCR). In total, 371 clinical respiratory specimens were tested for influenza/RSV with both assays. All of the 371 samples were valid in the UCT assay and clear amplification curves could be detected in positive samples (see Fig. 1). After discrepancy analysis, 1100 results were concordant while thirteen results remained discrepant (for detailed information see Supplementary Table 4), most discrepant samples were low positive specimens determined by low Ct value (> 35). Overall the UCT assay has a sensitivity and specificity for influenza A of 98.3% (CI: 94.1–99.8%) and 98.4% (CI: 96.0–99.6%), influenza B was 100% (CI: 91.6–100%), and 98.5% (CI: 96.5–99.5%), and RSV was 98.6% (CI: 92.3–100%), and 99.4% (CI: 98.2–100%) respectively (Table 5).

3.7. Workflow comparison

The workflow comparison of the UCT and the routine influenza and RSV assays is summarized in Table 6 (see also Supplementary Tables 5–8). The evaluation considered each of the manual steps and the time needed for sample preparation, DNA-extraction, PCR setup and reporting using 48 specimens. Overall, the UCT on the automated cobas 6800 System reduced the number of manual steps 58% (from 33 to 14), reduced hands-on time by 57% (from 74 min to 32) and simplified the testing. In addition, the number of multiple instruments and other equipment required was reduced (from 29 to 12).

4. Discussion

Optimal patient management and prevention strategies in LRTIs depend on fast and accurate diagnostics. In particular, the discrimination and/or exclusion of influenza viruses and RSV are mandatory, as these viruses account for the majority of LRTIs and require prompt prevention measures or quick administration of antiviral drugs (Rappo et al., 2016; Rogan et al., 2017; Schulert et al., 2013; Xu et al., 2013). However, strong yearly variation in intensity (ECDC, 2018) and disease burden characteristics (for example, emerging, pandemic strains like H1N1 in 2008 (Cheng et al., 2012)) do exist, posing a fluctuating test burden to a diagnostic molecular lab (Mathews et al., 2009). Furthermore, in regularly recurring global outbreaks, reliable high-throughput molecular test systems which cover a high number of specimens in parallel are needed (Ling et al., 2018).

In this study, we used the open mode of a high-throughput and fully

Table 5
Clinical sensitivity and specificity for influenza A, influenza B, and RSV after resolution of discrepant results.

	UCT Flu + RSV result / reference test after resolution				Sensitivity [95% CI] Clopper - Pearson	Specificity [95% CI] Clopper - Pearson
	pos/pos	pos/neg	neg/pos	neg/neg		
Flu-A	118	4	2	247	98.3% (94.1–99.8%)	98.4% (96.0–99.6%)
Flu-B	42	5	0	324	100% (91.6–100%)	98.5% (96.5–99.5%)
RSV	69	1	1	300	98.6% (92.3–100%)	99.7% (98.2–100%)

CI, Confidence Interval; Flu-A, influenza A; Flu-B, influenza B; RSV, Respiratory Syncytial Virus; pos, positive; neg, negative.

Table 6
Summary of workflow comparison of routine LDTs and **cobas** 6800 (48 specimens).

Parameters	RSV and Influenza A/B-assay (Extraction: NucliSENS EasyMAG, PCR: LC 480)	cobas 6800 Inf/RSV
No. of manual steps*	25 (37)	14
Hands-on time (sec)*	3068 (4443)	1921
Number of reagents and consumables needed	22	11
No. of hardware equipment needed	7	1

*Figures in brackets refer to steps and time needed for the setup of both routine LDT-assays (two EasyMAGs and two LC 480 necessary).

automated PCR platform (the cobas® 6800/8800 System) to validate a multiplex PCR assay for the simultaneous detection and differentiation of influenza A, influenza B and RSV (UCT assay) using commercially-available primer probes. The system demonstrated favourable performance for the management of different parameters including viral and non-viral targets (e.g., hepatitis B and C virus, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*) in several studies (Vermehren et al., 2017; Maasoumy et al., 2017; Yao et al., 2018; Van der Pol et al., 2019) and it is able to generate up to 960 results in an 8-hour shift. (Cobb et al., 2017).

All reagents used in this study within the UCT assay workflow (nucleic acid extraction chemistry, reverse transcription, multiplex PCR and full process control) are IVD grade and ready to use except custom primer and probes which were added to the master mix cassette (Boujnan et al., 2018). The analytical sensitivity (LoD) of the UCT assay was first analyzed by cell culture passaged virus using retrieved swabs. The UCT assay showed a LoD ranging from 0.009 to 0.202 TCID50/mL for influenza and RSV, respectively (Table 2). These values are in accordance with published sensitivity of other (CE/FDA cleared) molecular assays, such as the Aries Flu-A/B & RSV (Luminex Corp.) (Juretschko et al., 2017), the XPert Xpress Flu/RSV (Cepheid) (Chen et al., 2018) or the cobas® Flu-A/B & RSV on the cobas® Liat platform (Roche Molecular Systems) (Ling et al., 2018). The run-to-run variability of the UCT assay was low (< 1.1 Ct at 3× and < 0.6 Ct at 10× LoD) thus demonstrating excellent reproducibility, which can be attributed to both stable on-board chemistry and low variation due to complete automated workflow.

The existence of many different virus strains and subtypes (especially for Influenza A) makes inclusivity an ongoing concern in diagnostic testing (CDC, 2018b). In this study, the custom primers/probes were first analysed *in silico* using GISAID sequences for influenza A (human n = 6,643 and H5 avian n = 43) and influenza B (n = 2,934). The result demonstrated broad coverage of all human influenza strains circulating in 2018. Only H9N2 and H7N9 were predicted to be strongly delayed in Ct value. These *in silico* data were confirmed by the correct detection of the UCT assay of 63/63 external quality assessment panels samples (including H1N1, H1N1_09, H3N2, H5N1, H5N8 and H7N9). As predicted *in silico*, H7N9 was detected in panel samples, but at late Ct values and a strong delay compared to H7N9 specific primers set (Kile et al., 2017) was observed indicating increased LoD. No analytical interference or cross-reactivity of the UCT assay was observed with 28 other common viral and bacterial respiratory organisms tested. These data demonstrate the overall robust and broad coverage of the primer/probe design of the UCT assay.

A total of 371 clinical samples were analyzed and the results were compared with the current LDT workflow using a CE IVD approved assay. The UCT assay demonstrated good sensitivity (98.3%, 100% and 98.6% for influenza A, influenza B and RSV respectively) and specificity (98.4%, 98.5% and 99.7% for influenza A, influenza B and RSV respectively). These values are in accordance with published multi-assay comparisons (Banerjee et al., 2018; Ling et al., 2018; McMullen et al., 2017). Of note no invalid results occurred with the UCT, so there was no need for retesting which can reduce test efficiency (Ling et al., 2018). Moreover in this study the automated workflow with the UCT assay (cobas 6800) required significantly less manual steps (- 58%) and less hands-on time (- 57%) in comparison to routine laboratory methods using the EasyMAG-system for nucleic acid extraction and the LC 480-instrument for PCR. This result is in line with previous studies, showing a lower overall hands-on time compared with traditional molecular workflows (Cobb et al., 2017, Marlowe et al., 2017) while offering increased throughput (up to 960 results in an 8-hour shift for the cobas 8800). Furthermore, the improvement of the laboratory workflow (reduced manual steps) also leads to an overall lower risk of contamination and errors. A limitation of this study is that only nasopharyngeal swabs in UTM collection media were tested, so further studies are needed to validate the use of other collection media and alternative respiratory sample types, such as broncheoalveolar lavage.

5. Conclusion

In this study we evaluated the performance of a custom assay for the multiplex detection of influenza A/B and RSV (UCT) on a high-throughput molecular system (cobas® 6800/8800). The primers and probes of the UCT assay demonstrated broad coverage and the analytical sensitivity in nasopharyngeal swabs was similar to CE cleared IVD assays used in routine workflows while offering higher throughput capabilities and reduced hands-on time, both features which are of major importance for diagnostic laboratories facing seasonal influenza or outbreak scenarios.

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Declarations of interest

UE and ML received speaker honaria and related travel expenses from Roche Diagnostics.

GS is employee of Roche Molecular Systems.

All other authors had no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2019.03.015.

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